AMENDMENTS TO SPECIFICATION

On page 5, please delete the heading "Brief Description of the Drawings" and paragraphs [0013] to [0018] in their entirety.

Replace the following paragraphs as indicated below:

[0035] The problem of aggregate formation of recombinant PDE polypeptides was overcome in the present invention by generating a polypeptide comprising a PDE polypeptide sequence with the exception of the PDE4A polypeptide sequence, with an amino-terminal deletion, said polypeptide exhibiting decreased aggregate formation~~, as shown in Figures 2 and 3~~. These polypeptides had a specific activity that is comparable to the specific activity of the native recombinant proteins~~, as is shown in Figure 1~~. In a preferred embodiment, the invention provides a polypeptide comprising a PDE polypeptide sequence, with an amino-terminal deletion wherein the proportion of non-aggregated PDE in the total protein preparation is from 55 %, preferably from 60%, more preferably from 65%, most preferably ~~perferably~~ from 68% to 100%, preferably to 90 %, more preferably to 80 %, most preferably to 70% of total protein, as determined by the quantitation of eluted fractions from a gel filtration of the PDE fractions. Quantitation was performed as follows: Equal volume aliquots of fractions from Size Exclusion Chromatography, run as hereinbefore described, were analyzed by SDS-PAGE (one gel per run and isoform~~, Figure 3~~). Optical densities of Coomassie stained PDE were integrated as follows: After electrophoresis the Coomassie stained polyacrylamide gel was imaged by a video imaging system ~~(Figure 1)~~. Optical densities of PDE bands were integrated using a Macintosh computer and the public domain software "NIH Image", version 1.61 (developed at the U.S. National Institutes of Health and available from NIH or on the NIH website at http://rsb.info.nih.gov/nih-image/). The integrated arbitrary units per PDE band as returned by the software reflect the relative PDE

concentrations within the original pools. Integrated densities from PDE bands of fractions 2-4 were added up, giving a relative amount of aggregated PDE, PDE_{agg} ~~(Figure 3)~~. These fractions contained molecules of at least 2 Mio Daltons. Similarly, integrated densities from PDE bands of fractions 5-11 were added up, giving a relative amount of non-aggregated PDE, $PDE_{non\text{-}agg}$. In fractions 5-11, molecules in the range of 0.3 Mio Daltons eluted. Relative amount of total PDE, PDE_{total}, was given by the sum of PDE_{agg} and $PDE_{non\text{-}agg}$. The percentage of non-aggregated PDE was given by the ratio of $PDE_{non\text{-}agg}$ and PDE_{total}, multiplied by 100.

[0045] In another preferred embodiment of the present invention, the PDE polypeptide hereinbefore described exhibits decreased tubulin association, thus allowing to obtain purer PDE polypeptide preparations. To determine the decrease in tubulin association, the ratio of PDE molecules per tubulin molecules in the preparation of PDE polypeptide was calculated by quantitation of SDS-PAGE. Preferably, the ratio of PDE molecules per tubulin molecules is at least 5, preferably at least 10, more preferably at least 15, even more preferably at least 20, most preferably at least 25 molecules of PDE per molecules of tubulin. The tubulin content of PDE preparations was determined as follows: Equal volume aliquots of fractions from SEC were analyzed by SDS-PAGE ~~(one gel per run and isoform, Figure 3)~~. Optical densities of Coomassie stained PDE and tubulin bands were integrated as described hereinbefore for PDE aggregation quantitation. Integrated densities from PDE bands of fractions 2-11 were added up, giving a relative amount of total PDE, PDE_{total}. Likewise, integrated densities from tubulin bands of fractions 2-11 were added up, giving $tubulin_{2\text{-}11}$, and thereby the relative amount of total tubulin, $tubulin_{total}$. The ratio of PDE and tubulin ~~(Figure 4C)~~ was identical with the ratio of the relative amount of total PDE4D, PDE_{tot}, and relative amount of total tubulin, $tubulin_{total}$, on a mass per mass basis.

[0067] Relative concentrations of Ni-NTA agarose purified isoform preparations were estimated by SDS-PAGE. Equal volume amounts of isoform preparations were applied to a gradient gel (4-12% NuPage; Invitrogen). After electrophoresis the Coomassie stained polyacrylamide gel was imaged by a video imaging system ~~(Figure 1)~~. Optical densities of PDE4D bands were integrated using a Macintosh computer and the public domain software "NIH Image", version 1.61 (developed at the U.S. National Institutes of Health and available from NIH or on the NIH website at http://rsb.info.nih.gov/nih-image/). The integrated arbitrary units per PDE4D band as returned by the software reflect the relative PDE4D concentrations within the original pools ~~(Figure 1, "amount")~~. Identities of PDE4D and tubulin bands had been verified by independent SDS-PAGE, excision of corresponding bands, trypsin cleavage and identification of tryptic peptides by MALDI-MS.

[0068] Activities of equal volume amounts of 10^6-fold diluted purified isoforms were determined by use of a commercial radioactive phosphodiesterase assay (cAMP-dependent phosphodiesterase [^{3}H] assay; Amersham Pharmacia Biotech), following the instructions of the manufacturer. The obtained arbitrary activity units reflect the relative PDE4D activities within the original pools ~~(Figure 1, "activity")~~.

[0069] Relative specific activities of PDE4D isoforms were calculated by dividing relative activity values by relative concentration values ~~(Figure 1, "specific activity")~~.

[0070] 50 µl of Ni-NTA agarose purified isoform preparation was injected into a Superose 12 size exclusion column (type PC3.2/30; Amersham Pharmacia Biotech), equilibrated with 50 mM TrisHCl pH 7.7, 100 mM NaCl, 0.5 mM MgCl2 at a flow rate of 0.1 ml/min at 4°C.

Chromatograms were recorded at 278 nm. Starting from the elution volume, the column eluate was collected as 50 µl fractions ~~(Figure 2)~~.

Quantification of aggregates and tubulin content

[0071] Equal volume aliquots of fractions from SEC were analyzed by SDS-PAGE (one gel per run and isoform~~, Figure 3~~). Optical densities of Coomassie stained PDE4D and tubulin bands were integrated as described above. Integrated densities from PDE4D bands of fractions 2-4 were added up, giving a relative amount of aggregated PDE4D, PDE_{agg} ~~(Figure 3)~~. Similarly, integrated densities from PDE4D bands of fractions 5-11 were added up, giving a relative amount of non-aggregated PDE4D, $PDE_{non\text{-}agg}$. Relative amount of total PDE4D, PDE_{total}, is given by the sum of PDE_{agg} and $PDE_{non\text{-}agg}$. The percentage of non-aggregated PDE is given by the ratio of $PDE_{non\text{-}agg}$ and PDE_{total}, multiplied by 100 ~~(Figure 4B)~~.

[0072] Similarly, integrated densities from tubulin bands of fractions 2-11 were added up, giving $tubulin_{2\text{-}11}$, and thereby the relative amount of total tubulin, $tubulin_{total}$. The ratio of PDE and tubulin ~~(Figure 4C)~~ is identical with the ratio of relative amount of total PDE4D, PDE_{tot}, and relative amount of total tubulin, $tubulin_{total}$.